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# Diversity Of Endophytic Fungal Assemblages In Submerged Musk Grass An Aquatic Plant Of Chara delicatula L.

**J. Rameshkumar, G. Venkatesan**
P.G. and Research Department of Botany, Mannai Rajagopalaswami Government Arts College, Mannargudi-614001, Thiruvarur -District, Tamil Nadu, India.

## ABSTRACT

Endophytes were isolated in culture from the surface of water conduct photosynthetic leaf tissues during six month continuous growing seasons. A total of 33 representing species were recovered from a total of 600 (every month/100 bits) plant leaves tissues segments of musk grass; stonewort in the submerged aquatic plant of *Chara delicatula*. Although isolation frequency was low, endophytes were phylogenetically diverse groups and species-rich. Compactly among the six months, a continuous study in leaf sampled and a pond revealed that frequency isolation and diversity did differ significantly between collection periods, among species are slightly varied or in the maturation, between leaves ages. These plants were collected from a fresh-water pond in winter season at Mannargudi in Tamil Nadu. Among these three belongs to Ascomycetes. Five fungal species belonging to Coelomycetes and twenty Hyphomycetes beside to five sterile forms. Endophytic fungi were studied shows that some of occupying the leaves tissues.

**Keywords:** Aquatic plant, *Chara* sp., fungal endophytes, tissues specificity.

## INTRODUCTION

Microorganism live in association with the plant (endophytic microbes) is commonly observed in nature (Bacon and White 2000). Endophyte was coined by de Bary (1866) to define "Organisms that colonize internal plant tissues". They are commonly found in coniferaceae, Gramineae, (Okane *et al*., 2001). Estimations of the total number of fungi have major implications for systematics, resources and classification (Hawksworth 1991). In contrast, an updated estimate of fungal diversity showed that the fungal species ranged from 2.2 to 3.8 million worldwide (Hawksworth and Luecking 2017).This fungus can also occur as endophyte of marine algae (Tarman *et al*., 2012). Fresh water plants endophytic fungi (Dustin *et al*., 2014; Young Hyun *et al*., 2015; Rajagopal *et al*., 2018; Venkatesan and Ramesh kumar 2020) Fungal endophytes have also been reported from marine algae (Cubit 1974; Andrew *et al*., 2013; Venkatachalam *et al*., 2015), mosses and ferns (Petrini 1986; Tao Zhang *et al*., 2013; Andreea *et al*., 2018), and mangroves (Suryanarayanan *et al*., 1998; Arfi *et al*., 2012; Li *et al*., 2016). Endophytic microbes, especially endophytic fungi have been recognized as a potential source of the diverse array for bioactive secondary metabolites (Tan and Zou 2001). Seaweeds host a number of endophytic organisms, including bacteria (Apt and Gilbor 1989) algae (Andrews 1977). In the course of time, the definition of endophytes has evolved to embrace those fungi which have lengthy epiphytic phase and latent endophytic phase (latent pathogens), that may live symptom less in their hosts for some time in their life (Petrini 1991). Species richness and distribution of fungal endophytes vary in relation to the collection site (Petrini 1987, Sieber-Canavesi and Sieber 1987). The present study has been conducted to understand the association of fungi with leaves of *Chara delicatula* aquatic plant.

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## MATERIALS AND METHODS

### *Taxonomy and Collection of plant*

*Chara delicatula* are a small group of plants technically known as charaphyceae. These aquatic algae look like regular vascular plants because they form stem like, leaf like, and root like structures. *Chara* is gray green, gritty texture, a musky and whorls of needle like structure that resemble leaves. Although, musk grass or stonewort can be thought of as green macro algae. Musk grass grows submerged in freshwater ponds or lakes, especially in clear hard water. *Chara* plant was encrusted with lime (calcium carbonate). The host plant was collected from Mannargudi in Tamil Nadu, South India. Unlike most green algae, stonewort has distinctive macroscopic features, filamentous and they are small plant, heavily calcified as to make the plants rough to the tough and even brittle. The green thallic originated by produces node and intermodal, whorls of so-called leaves with limited growth arise from nodal cells and so do branches.

Leaf samples were collected from healthy between two whorls of ‘‘leaves” each originating from a node, is a single intermodal cell was transported to the lab in closed sterile polythene bags. Randomly select One hundred tissue segments (Filamentous leaves) from host species in every month. They were processed within 24 hours of collection (Fisher and Petrini 1987).

### *Surface sterilization of leaves*

One hundred filaments whorled branched leaves bits of 0.5 $cm^2$ each were cut and surface sterilized by the method of Suryanarayanan *et al*., (1998). The samples were washed in running water, dipped in 70% ethanol for 5 seconds, immersed in 4% NaoC1 for 90 seconds and then washed in sterile water for 10 seconds or three times. The sterilized samples were placed on PDA medium amended with antibiotic contained in Petri dishes.

### *Incubation, isolation and identification of endophytes*

Ten segments were placed on PDA medium contained in a Petri dish. The Petri dish was sealed with Parafilm$^{TM}$ and incubated in a light chamber at 26±1$^{o}$C for 5 -21 days (Bills and Polishook 1992, Suryanarayanan 1992). The light regimen given was 12 hours light followed by 12 hours darkness. The fungi that grew from the segments were periodically observed and the endophytes were identified.

### *Statistical Analysis*

#### *a. Species evenness index and species richness index (E5, R1)*

The species evennesss (E5, modified Hill’s rtio) and richness (R1, Margalef’s index were calculated as described by Ludwig and Reynolds (1988) using the software provided by the John Wiley and Sons, SPDIVERS. BAS.

#### *b. Diversity index (Fisher’s alpha)*

Diversity index was calculated using the method of Fisher (*et al*., 1943).

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***c. Relative percentage of occurrence of each group of fungi (RPO)***

Relative Percentage of Occurrence (RPO) of each group (*viz.* Ascomycetes, Coelomycetes, Hyphomycetes and sterile forms) of fungal species in each month of plant species was calculated as follows:

$$RPO = \frac{\text{Total colonization frequency of one group}}{\text{Total colonization frequency for all the Groups of fungi}} \times 100$$

## RESULTS AND DISCUSSION

*Chara* species can appear to be a plant that would produce flowers and seeds however it is actually a multi-cellular macro alga. This macro- alga has no true "leaves", only branches and branch lets. A regard of musk grass aquatic plant endophytes was isolated in six-month continuous studied. For *Chara* plant, I personally obligate recorded 436 endophyte isolates during six months from 600 leaf segments. In most of the cases, each tissue segment was infected by more than one fungal species (Multiple infections) Arnold *et al*., 2000. Several ecological groups of plants such as mangroves (Suryanarayanan *et al*., 1998; Kumaresan *et al*., 2000), halophytes (Suryanarayanan and Kumaresan 2000), angiosperm parasite and its hosts (Suryanarayanan et al., 2000) and trees of tropical forest (Suryanarayanan *et al*., 2002, 2003, 2004; Murali *et al*., 2007) have been studied for their endophyte assemblages. Based on their studies on two neotropical tree host, Arnold *et al*., (2000) concluded that tropical endophytes are hyperdiverse and the figure of 1.5 million "may markedly underestimate fungal diversity". A few studied in aquatic habitat from southern India (Rajagopal *et al*., 2018; Venkatesan and Ramesh kumar, 2020; Venkatesan and and Arun, 2020); However, several aquatic plants have not been studied for their endophyte association. Hence this study was carried out *Chara* species of aquatic plants screened for the presence of fungal endophytes harboured in their whorled leaves. A total of 33 fungal species were obtained during the study periods. The endophytes included three Ascomycetes, five Coeleomycetes, twenty Hyphomycetes and five sterile forms. A few Ascomycetes such as *Chaetomium* sp, *Sporormiella* sp. were isolated. Intriguingly, a few coprophilous genera (*Chaetomium*, *Sporormiella* etc.) have been reported as endophytes from several hosts (Petrini, 1986). A Coeleomycete member *Colletotrichum gloeosporioides*, *Phyllosticta capitalensis* was isolated dominated the endophyte assemblages of during the study. This fungus is ubiquitous forms and contributes substantially to the endophyte assemblage of the leaves of many plants (Suryanarayanan *et al*., 2002, 2003, 2011; Murali *et al*., 2011). In addition, some hyphomycete fungi including *Alternaria* sp. *Cladosporium* sp, *Drechslera* sp. is isolated as endophytes (Table 1). No basidiomycete member was present as an endophyte. Hence, members of this class are rarely encountered as endophytes (Petrini 1986). These endophytes were recorded to 17 species and 47 colonization were isolated in August'19, 20 fungal species and 60 colonization isolated in September'19, 21 fungal species and 61 colonizes were isolated in October'19, 27 fungal species and 95 colonizes were isolated in November'19, 26

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fungal species and 91 colonization were isolated in December'19, and 23 fungal species and 82 colonization were recorded in January'20.

**Table 1. Fungal endophytes isolated from the filamentous leaves of *Chara delicatula* during different months.**

| Sl.No. | Endophytes | Months | | | | | |
|---|---|---|---|---|---|---|---|
| | | Aug-19 | Sep-19 | Oct-19 | Nov-19 | Dec-19 | Jan-20 |
| | **Ascomycetes** | | | | | | |
| 1 | Chaetomium globosum | 3 | 3 | 5 | 5 | 7 | |
| 2 | Sporormiella sp. 1 | | | 4 | 6 | 6 | 8 |
| 3 | Talaromyces sp. 1 | 2 | 2 | 3 | 4 | 5 | 6 |
| | **Coelomycetes** | | | | | | |
| 4 | Chaetomella sp. 1 | | | | 8 | 9 | |
| 5 | Colletotrichum gloeosporioides | 7 | 9 | 8 | 9 | 8 | 9 |
| 6 | Phoma herbarum | 2 | 2 | 1 | 2 | 2 | 1 |
| 7 | Phomopsis sp. 1 | 6 | 5 | 4 | 6 | 6 | 7 |
| 8 | Phyllosticta capitalensis | 9 | 8 | 9 | 9 | 8 | 7 |
| | **Hyphomycetes** | | | | | | |
| 9 | Acremonium strictum | | 2 | 2 | 1 | | |
| 10 | Alternaria alternata | 1 | 3 | 1 | 1 | 1 | |
| 11 | Aspergillus sp. 1 | 3 | 4 | 2 | 3 | 3 | 3 |
| 12 | Aspergillus flavus | 1 | 3 | 1 | 6 | 4 | 4 |
| 13 | Aspergillus niger | 2 | 1 | 3 | 3 | 5 | 3 |
| 14 | Aureobasidium pullulans | 1 | 2 | | | 3 | 1 |
| 15 | Cladosporium cladosporioides | 3 | 2 | 2 | 5 | 7 | 5 |
| 16 | Curvularia lunata | 1 | 2 | 2 | 2 | 3 | 8 |
| 17 | Curvularia tuberculata | | | | 1 | 1 | |
| 18 | Beltrania rhombica | | 1 | | | 1 | 1 |
| 19 | Drechslera hawaiiensi | | | 1 | 1 | | |
| 20 | Fusarium oxysporum | 2 | 4 | 4 | 5 | 3 | 7 |
| 21 | Fusarium solani | | | | 1 | | 1 |
| 22 | Nigrospora oryzae | 1 | | 1 | | | 1 |
| 23 | Paecilomyces fumosoroseus | | | | 1 | | |
| 24 | Penicillium sp.1 | 2 | 4 | 2 | 6 | 1 | 3 |
| 25 | Penicillium sp. 2 | | | 1 | 3 | 1 | 2 |
| 26 | Penicillium sp. 3 | | | | 1 | | 1 |
| 27 | Trichoderma tuberculatum | | 1 | 2 | 1 | 1 | 1 |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 28 | Wardomyces anomalus | | | | | 1 | |
| | **Sterile forms** | | | | | | |
| 29 | CD 1 | 1 | 1 | 3 | 3 | 2 | 1 |
| 30 | CD 2 | | | | | 1 | 1 |
| 31 | CD 3 | | | | | 1 | |
| 32 | CD 4 | | | | 1 | 1 | 1 |
| 33 | CD 5 | | 1 | | 1 | | |
| **Total No. of Species** | | 17 | 20 | 21 | 27 | 26 | 23 |
| **Total No. of Isolates** | | 47 | 60 | 61 | 95 | 91 | 82 |

**Table 2: Number of species, isolates, and diversity of endophyte fungi from the *Chara delicatula* during six months.**

| | **Aug'19** | **Sep'19** | **Oct'19** | **Nov'19** | **Dec'19** | **Jan'20** |
|---|---|---|---|---|---|---|
| **Total no. of species** | 17 | 20 | 21 | 27 | 26 | 23 |
| **Total no. of isolates** | 47 | 60 | 61 | 95 | 91 | 82 |
| **Total no. of segments** | 100 | 100 | 100 | 100 | 100 | 100 |
| **R1(Margalef's)** | 4.16 | 4.64 | 4.87 | 5.71 | 5.54 | 4.99 |
| **H' (Shannon Index)** | 2.55 | 2.77 | 2.81 | 3.03 | 2.98 | 2.83 |
| **E5 (Hill's Ratio)** | 0.98 | 1.04 | 1.03 | 1.03 | 1.02 | 1.00 |
| **Fisher's Alpha** | 9.57 | 10.51 | 11.33 | 12.58 | 12.16 | 10.62 |
| **Relative Percentage of Occurrence (RPO) of each group of fungal species** | | | | | | |
| **Ascomycetes** | 11.7 | 10 | 14.2 | 11.1 | 11.5 | 8.6 |
| **Coeleomycetes** | 23.5 | 20 | 19 | 18.5 | 19.2 | 17.3 |
| **Hyphomycetes** | 58.8 | 60 | 61.9 | 59.2 | 53.8 | 60.8 |
| **Sterile forms** | 5.8 | 10 | 4.7 | 11.1 | 15.3 | 13.04 |

The procedure for inoculation of endophytes from leaves of these age classes was exactly like the mature leaves more densely colonized by endophytes when compared with younger (August 2019 to January 2020) leaves. Similarly, the number of endophyte species also increased during the study periods (Table 1-2 and Fig 1).

**Figure 1. Number of fungal endophytes and species isolated from the leaves of *Chara delicatula* during study Period.**

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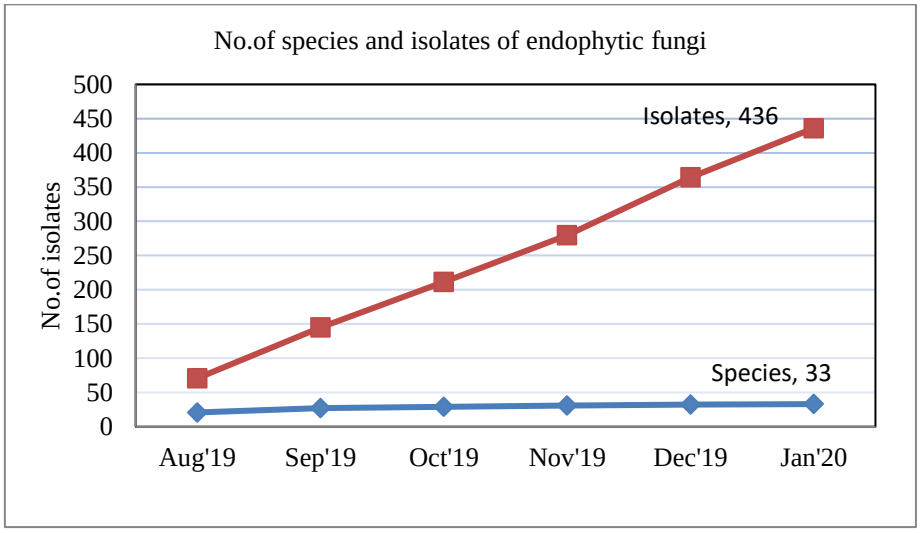
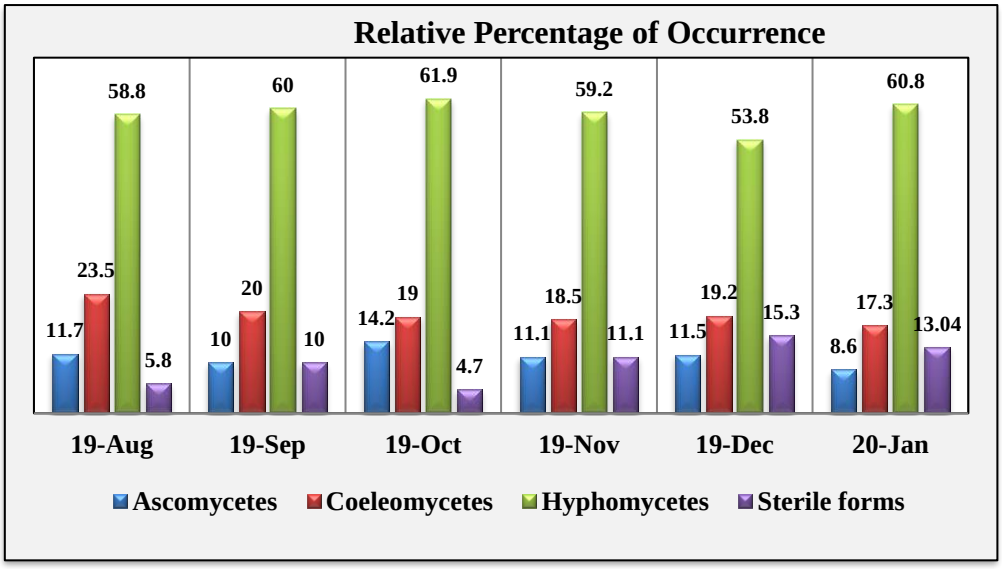



**Figure 2. Relative Percentage Occurrence (RPO) of endophytes belonging to different groups of fungi from *Chara delicatula* during study Period.**




This study shows that hydrophyte plants harbour endophytic fungi in their leaves and to a greeted extent; these endophytes are different from those that occur on the leafage of these hydrophytes. Also, a few previous studies report from southern India for freshwater plants endophyte (Rajagopal *et al*., 2018; Venkatesan and Ramesh kumar, 2020; Venkatesan and Arun, 2020). Many reported freshwater ascomycetes are an ecological assemblage of fungi that occur on submerged or partially submerged substrates in aquatic habitats (Shearer 1993; Raja *et al*., 2005). The present study suggested that the endophyte diversity. For the first time, a musk grass aquatic plant was screened for endophytes. Coleomycetes, hyphomycetes, ascomycetes and sterile forms were present as endophytes. Coleomycete members contributed more to the endophyte assemblage of the plant. To our knowledge, this is one of the first reports dealing with the mycoflora of aquatic plant. Further studies are warranted can throw more light on climatic and nutrient factors and endophytic fungi would elucidate their importance in the freshwater plant. Similar information for most fungi in aquatic plants (Venkatesan and Ramesh kumar 2020; Venkatesan and Arun 2020). A total of

33 fungal species and 432 colonizes were recovered simultaneously during study periods. These endophytic fungal species belonging to 11.7%, 10.0%, 14.2%, 11.1%, 11.5% and 8.6% of ascomycetes, 23.5%, 20%, 19%, 18.5%, 19.2%, 17.3% of coelomycetes, 58.8%, 60%, 61.9%, 59.2%, 53.8%, 60.8% of hyphomycetes, 5.8%, 10%, 4.7%, 11.1%, 15.3%, 13.4% of sterile forms are recorded (Tables 1-2). Most of the species were isolated in fewer colonies or spores. The total number of colonization has been different significantly among endophytic fungi from young to mature leaves. Hence, a total of 72% in the diversity of fungi was isolated from 600 segments in tissues of a single plant. The species diversity of endophytic fungi in an age of leaf tissues were increased higher colonization for the monthly (Tables 1-2 and Fig. 1-2). Fungal endophyte assemblage belonging to hyphomycetes taxa were highest colonization frequency to all months than coelomycetes and ascomycetes (Fig. 2). Coelomycetes dominated the endophyte assemblage. *Colletotrichum* sp., *Phomopsis* sp., and *Phyllosticta* sp. showed the highest colonization frequency during all months, as well as hyphomycetes species *Fusarium* species were dominated (Table 1). The species richness or assumes the abundance of the species diversity index (Fisher's alpha) of the fungal assemblage of the plant was increased such as 9.57,10.51,11.33,12.58,12.16 of during five months for except January'2020 and Margalef's of the calculative showed in 4.16, 4.64, 4.87, 5.71, 5.54. (Table 2). Although, the plant-microbial assemblage was found in the fungal species diversity is increased in the age of leaves. Also, the richness indexes (R1), Hills ratio (E5) and Shannon Index (H') in this host of tissues were similarly found (Table 2). A few fungi which failed to sporulate were designated as "mycelia sterile", can be become identified in later with different incubation such as sporulation in UV, so for colony characteristics, the mycelia were transferred into PDA agar media.

## CONCLUSION

In this research, *Chara* plant species of host plant were found to be monthly varied colonized by endophytes. These endophytes were isolated in 33 fungal species, endophytic distribution in leaves of *Chara delicatula* has continuous studied in winter season. The number of fungal species, and colonization were diversely isolated; but in this fungal species nearly similar by monthly. It may be colonies increased in mature tissues from younger. Also, these kind of submerged plants occur beneath the water surface, with occasional floating leaves that protrude only a small distance from the water. Hence, it's too may be cause the colonization to be different.

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